Muhammed, Amir and James Trosko. Oak Ridge National Laboratory, Oak Ridge, Tennessee. A search for photoreactivating enzymes in D. mel. extracts.

Photoreactivating (PR) light given as a post-treatment increases embryo survival and adult fertility, and decreases recessive lethal mutation rate after low doses of ultraviolet (UV) radiation to polar cap cells of Drosophila (Meyers, 1951, Genetics 36:565; Browning and Allenburg, 1962, Genetics 47:361). Also, UV-induced phenocopies in pupae can be photoreactivated (Perlitsch and Kelner, 1953 Science 118:165). The question of whether these phenomena were dependent on a photoreactivating enzyme similar to that in yeast and Escherichia coli (Rupert, 1960, J. Gen. Physiol. 43:573) was investigated. Eggs, larvae, pupae, and adult Drosophila were grown aseptically and homogenized in cold 0.05 M phosphate buffer (pH 7.3). The homogenates were sonicated for 20 sec. to rupture cells and were then filtered through miracloth to remove chitinous debris.

The photoreactivating enzyme activity of different extracts was tested by their ability to photoreactivate UV-irradiated transforming DNA as described earlier (Muhammed, in press, J. Biol. Chem.). A large excess of calf thymus DNA was added to the reaction mixtures in order to protect the transforming DNA against DNases. The results of a typical experiment are summarized in Table 1.

Table 1. Photoreactivating enzyme activity of various extracts from Drosophila.

		No of Streptomycin Transformants		Ratio of PR/dark
No.	Extract	Dark Control	PR	Transformants
1	Growth medium	1081	1065	0.99
2	Eggs	1201	1220	1.02
3	Larvae, 3-day	1112	1184	1.06
4	Larvae, 7-day	930	899	0.97
5	Pupae, 1-day	1093	1129	1.03
6	Pupae, 2-day	1109	1110	1.00
7	Adult	1373	1323	0.96

No photoreactivating enzyme activity was detected under the assay conditions used in these experiments. It was realized that the lack of PR activity in the extracts might be due to the presence of some substances which could strongly inhibit the enzyme activity. When purified yeast PR enzyme was added to the extract, however, there was no loss in activity, indicating the absence of any inhibitory material in Drosophila extracts. This suggests that the reported photoreactivation phenomena in Drosophila are mediated through an indirect effect (Jagger and Stafford, 1965, Biophys. J. 5:75). Another possibility is that the photoreactivating enzyme is present in too small amounts, or that the enzyme in Drosophila is of a different type than the one in micro-organisms, and is not detectable by the assay used in this work. The extracts were diluted to a final protein concentration of approximately 0.5 mg/ml in 0.04 M phosphate buffer (pH 7.2). The reaction mixture contained 0.2 ml of UV-irradiated H. influenzae transforming DNA (10 γ/ml, 1% survival 2480 ergs/mm^2), 0.2 ml calf thymus DNA (1.80 mg/ml), and 0.4 ml of the diluted extract. 0.4 of the mixture was kept as a dark control, and the remaining photoreactivated for 20 minutes. Conditions for photoreactivation and assay of transforming DNA have been previously described (Muhammed, in press, J. Biol. Chem.). (Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation and American Cancer Society Fellowship to J. E. Trosko, PF-253)